Botrytis Rot of BELL PEPPERS

Marketing Research Report No. 754

Agriculture Research Service
UNITED STATES DEPARTMENT OF AGRICULTURE

CONTENTS

	Page
Summary	II
Review of previous work	1
Materials and methods	2
Results and discussion	3
Effect of temperature on growth rate of *Botrytis* on nutrient agar	3
Effect of temperature on infection from inoculation of wounded peppers	4
Effect of temperature on natural infection	5
Effect of temperature on infection from artificial inoculation of nonwounded peppers	6

SUMMARY

The rate of decay when *Botrytis cinerea* was inoculated into bell peppers through a wound and the rate of growth when the fungus was placed on nutrient agar increased as the temperature was increased between 32° and 70° F. The only exception was at 50° when decay developed faster than it did at 55° and in some tests at 60°.

Natural infection similar to that observed on commercial shipments of peppers occurred in tests without the aid of wounds. Peppers held about 3 weeks developed practically no botrytis rot at 55° and 50° F., an intermediate amount at 45°, and most at 40°. This indicates that low, but nonfreezing, temperatures provide the predisposing condition for the rot.

In a series of inoculation tests, spores were dusted on the nonwounded surface of bell peppers after holding at 32° F. for 0, 4, 8, 12, 16, and 20 days. After the inoculation, each lot in closed plastic trays was moved to 55° where condensation water developed and was maintained for 5 days to hasten spore germination. In these tests botrytis rot was directly related to the holding period; the rot increased as the holding period was increased.

Washington, D.C. Issued November 1966

Botrytis Rot of BELL PEPPERS

By Lacy P. McColloch, formerly [1] *senior plant pathologist,* and William R. Wright, *plant pathologist, Market Quality Research Division, Agricultural Research Service* [2]

Gray mold rot, caused by *Botrytis cinerea* Pers. ex Fr., occurs each year during the marketing of bell peppers. Such decay occurs as numerous lesions on the pods. Although peppers are readily infected through wounds such as broken stems and skin breaks, these lesions usually develop without the aid of visible skin breaks.

Although entire carloads have been dumped because of this disease, serious losses do not consistently occur. The intermittent nature of the losses together with the wide distribution of the *Botrytis* fungus as a saprophyte in nature suggests that infection depends on the presence of one or more predisposing factors.

The purpose of this study was to determine the factor or factors that predispose peppers to infection and decay. This was accomplished through studies on the effect of temperature on growth rate of *Botrytis* on nutrient agar, on the rate of decay when the fungus was inoculated into bell peppers through a wound, on percentage of pods that developed natural infection, and on infection from inoculation of nonwounded peppers.

REVIEW OF PREVIOUS WORK

Botrytis rot, or gray mold rot, occurs on a wide variety of fruits and vegetables, and a number of papers have been published on the subject. Very little, however, has been published on botrytis rot of bell peppers.

---

[1] Retired Dec. 30, 1965.

[2] Acknowledgment is made to W. H. Redit and M. J. Payne, Market Quality Research Division, for assistance with relative humidity control; and to Walter Dayette, formerly a member of the Division, who assisted with setting up experiments and obtaining data.

Apparently Peltier,[3] in 1912, was the first to report *Botrytis cinerea* as a fruit rot of peppers in the field.

In 1917 Brooks and Cooley [4] reported that the rate of growth of *Botrytis* on agar increased directly as the temperature increased.

In 1930 Lauritzen and Wright [5] published the results of a study on the storage of peppers. They described the rot caused by *B. cinerea* on cold-stored bell peppers and differentiated it from freezing injury. They reported that pods inoculated through wounds with *Botrytis* became infected at temperatures from 32° to 55° F. They also made studies on the effect of temperature, humidity, and length of storage on the natural occurrence of botrytis rot. In one test they found no infection in 55° in 39 days, but they considered the low relative humidity (76 percent) to be the limiting factor. However, they thought that temperature might be a factor in susceptibility. They noted, for example, that, with similar humidities, decay was much higher at 40° than at 50°. About 18 days was the minimum period required for natural infection to occur. At 40°, 50°, and 55°, infection increased as the humidity was increased from about 74 to about 95 percent. Since these investigators found more pods infected with gray mold rot at 40° than at 32° in the periods tested, they did not stress the weakening or chilling effect of low temperature as a predisposing factor in gray mold rot. On the contrary, they recommended 32° for successful storage. However, the highest humidity that they used at 32° was 90 percent, which is probably near or slightly below the lower limits of humidity required for *Botrytis* spore germination and infection.

In studying chilling injury and alternaria rot of bell peppers, McColloch [6] observed that low, but nonfreezing, temperatures appeared to predispose the pods to botrytis rot. Under those conditions several to numerous lesions of botrytis rot developed without the aid of skin breaks. This is typical of the disease found during marketing.

MATERIALS AND METHODS

Bell peppers used in the tests were of the California Wonder type. They were either locally grown or shipped in a normal transit period

[3] PELTIER, G. L. A CONSIDERATION OF THE PHYSIOLOGY AND LIFE HISTORY OF A PARASITIC BOTRYTIS ON PEPPER AND LETTUCE. Mo. Bot. Gard. Ann. Rpt. 23: 41–74. 1912.

[4] BROOKS, CHARLES, and COOLEY, J. S. TEMPERATURE RELATIONS OF APPLE-ROT FUNGI. Jour. Agr. Res. 8: 139–164. 1917.

[5] LAURITZEN, J. I., and WRIGHT, R. C. SOME CONDITIONS AFFECTING THE STORAGE OF PEPPERS. Jour Agr. Res. 41: 295–305. 1930.

[6] MCCOLLOCH, L. P. CHILLING INJURY AND ALTERNARIA ROT OF BELL PEPPERS. U.S. Dept. Agr. Market. Res. Rpt. 536, 16 pp. 1962.

from Florida, Texas, or New Jersey and purchased on the local wholesale market.

Mature-green peppers were used for the tests. They were washed with a mild detergent by a quick dip, to prevent water from entering the small opening in the blossom end of some pods, and were wiped dry. They were graded for size, shape, soundness, and firmness. Experimental lots were carefully selected for uniformity.

For inoculation purposes a heavy sporulating strain of *Botrytis cinerea* isolated from decaying bell peppers was used throughout the study. The fungus was grown on Thaxter's agar, an unoxidized potato dextrose, and was exposed to the light at room temperature. Spores for inoculating were harvested, when the cultures were about a week old, with an improvised vacuum collector. Collected spores were passed through a No. 60 screen to break up the clumps. Although collected *Botrytis* spores remained viable for 3 months or longer, those used in the tests were not held longer than 4 days.

RESULTS AND DISCUSSION

Effect of Temperature on Growth Rate of *Botrytis* on Nutrient Agar

Although not directly related to pepper infection and decay, the growth rate of *Botrytis* at various temperatures on nutrient agar is a useful step in studying the nature of infection and the method of control.

Both the rate of growth of a fungus on agar at a temperature range of 32° to 80° F. and the rate of decay when the fungus is inserted into wounds in the host held at similar temperatures have long been standard procedures for determining recommended protective refrigeration.

The rate of growth on Thaxter's agar was measured at 32°, 40°, 45°, 50°, 55°, 60°, and 70° F. Cultures held at 50° and below were grown in petri dishes of 150-mm. diameter. Cultures at temperatures above 50° were grown in 200-mm.-long Roux bottles to allow more space for fungus growth. A 4-mm. disk of inoculum, cut through the fungus mat and agar, was inverted on the agar surface at the center of each receptacle. The same depth of agar was used in each receptacle. In two experiments colony diameters were measured at 3, 5, and 7 days. In a third test, diameters were measured daily for 7 days.

The rate of growth of *Botrytis* on Thaxter's agar increased directly as the temperature increased from 32° to 70° F. (fig. 1). This is in agreement with published results of Brooks and Cooley.[7]

[7] See footnote 4, p. 2.

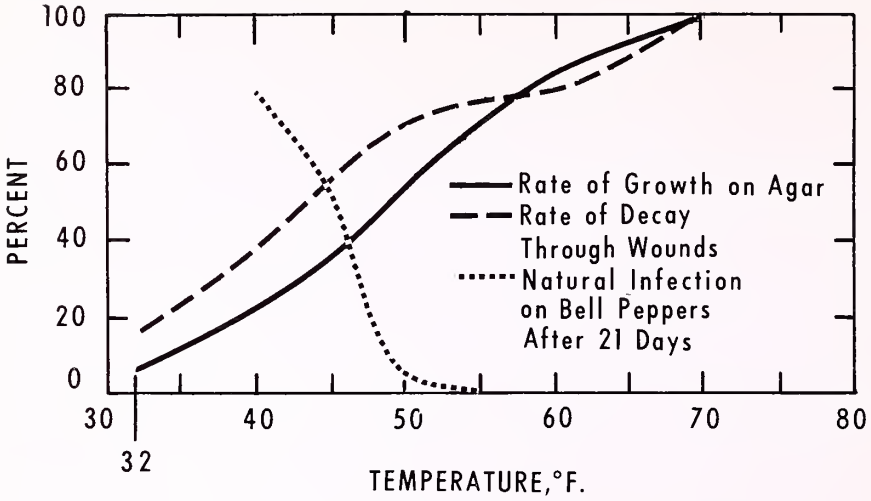

FIGURE 1.—Comparison of the effect of temperatures on the precentage of natural infection of *Botrytis cinerea* on bell peppers, the rate of growth of the fungus on nutrient agar, and the rate of decay when the fungus was inoculated into bell peppers through a wound.

Effect of Temperature on Infection From Inoculation of Wounded Peppers

The purpose of these tests was to determine the effect of temperature on the rate of decay where *Botrytis* was inoculated into bell peppers through a wound. Freshly harvested peppers were prepared for test as previously described. A uniform wound was made on each pod with a cork borer 4 mm. in diameter. A circular cut was made about halfway through the wall. A narrow scalpel was inserted obliquely under one side of the cut area and that portion was raised to form a pocket. The same cork borer used to wound the pods was then used to cut disks of the *Botrytis* fungus from cultures 4 to 6 days old. The disks were inverted and placed in the wound pockets.

In each of four experiments 12 to 20 inoculated pods were stored at each of seven temperatures—70°, 60°, 55°, 50°, 45°, 40°, and 32° F. They were placed in plastic trays covered by ventilated polyethylene bags. The diameter of the lesions was measured at 3, 5, 7, 9, 11, and 13 days. In a fifth experiment 9 pods were inoculated and held in similar trays at the same temperatures, but the rate of decay was measured daily for 13 days.

The decay rate was slowest at 32° and most rapid at 70° F. (fig. 1). With the exception of 50° the decay increased uniformly as the holding temperature was increased. In each of the tests the rate of decay

at 50° overtook that at 55° and in most tests that at 60° and continued to increase faster than that at 55° or in some instances 60°. Since this phenomenon did not occur when *Botrytis* was grown on agar, it seems logical that 50° is a borderline temperature for chilling injury and that the pepper pods were weakened enough to permit more rapid decay at 50° than at 55° and 60°.

At 32° to 70° F., with the exception of 50° F., as noted above, the rate of decay when *Botrytis* was inoculated into bell peppers through a wound (fig. 2) was similar to the rate of growth of the fungus on agar (fig. 1).

FIGURE 2.—Influence of temperature on rate of decay when a 4-mm. disk of inoculum was inoculated into bell peppers through a 4-mm. wound.

From these data it appears that botrytis rot could be held in check by refrigeration at 32° to 40° F. Yet, from observations of the disease during marketing, overrefrigeration was suspected as the predisposing factor.

Effect of Temperature on Natural Infection

In each of eight experiments about 15 pods of locally grown bell peppers were stored at each of four temperatures—55°, 50°, 45°, and 40° F. The peppers were held at a relative humidity of about 94

percent in single-layer fiberboard boxes each enclosed in a ventilated polyethylene bag. They were held for about 3 weeks.

Invariably the pattern of decay from natural inoculation was the same. There was little or no decay at 55° and 50° F., an intermediate amount at 45°, and the greatest amount at 40° (figs. 1 and 3).

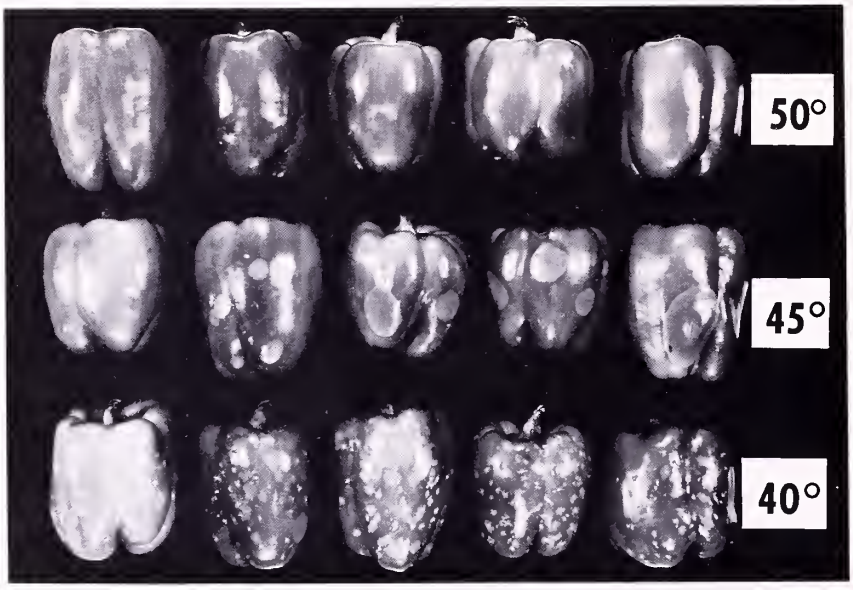

FIGURE 3.—Influence of temperature on the extent of natural infection of bell peppers by *Botrytis cinerea* in 21 days.

Effect of Temperature on Infection From Artificial Inoculation of Nonwounded Peppers

Although the foregoing data indicate that chilling injury of peppers is the principal predisposing factor in gray mold rot, there remained the unanswered question of the influence of a possible film of condensation water on the surface of the pods on infection and decay. The effect of chilling injury on the pods was more completely examined through a test in which the holding period at 32° F. was the only variable.

Uniform lots of peppers were dipped in 70 percent alcohol and drained dry. Six wound-free areas about three-fourths of an inch in diameter were outlined with indelible ink on each pod. The pods were held in closed plastic trays at 32° F. for 0, 4, 8, 12, 16, and 20 days. They were then inoculated and held at 55° and about 96 per-

cent relative humidity for 14 days. Inoculation was done by dusting spores on the surface of each encircled area with a small camel's-hair brush.

Condensation moisture on the pods at 55° F. gave optimum conditions for prompt *Botrytis* spore germination and for infection and decay on the inoculated pods. After the pods were inoculated, wet paper towels were placed in the plastic trays and the lids were closed to conserve the film of condensation moisture that developed when the peppers were transferred from 32° to 55°. Even the check lot (0 days at 32°) was held at 32° for 4 hours and then moved to 55° to create condensation moisture. The trays were covered with wet wrapping paper for 5 days to maintain the film of condensation moisture long enough for the *Botrytis* spores to germinate. The paper was then removed, but the lids were left on the containers throughout the holding period.

In about 4 days the *Botrytis* spores had germinated. Apparently sufficient toxic material was released by the germinated spores to cause death of the superficial tissues about the spores; this caused a dark-speckled appearance (fig. 4). Death of superficial tissues, however, did not permit infection through the unbroken skin if the pods were not weakened by low temperature. Thus, peppers in the check lot that were not held at 32° F. generally developed no infection; yet the spores

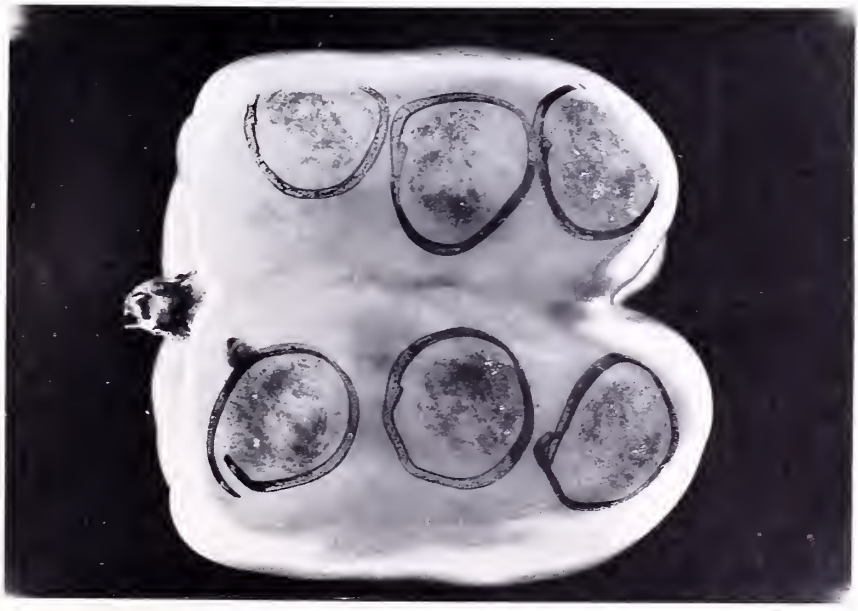

FIGURE 4.—Dark-speckled appearance caused by germinated *Botrytis* spores within the nonwounded, inoculated areas on nonchilled bell pepper.

were not dead. Spore counts showed that as many as 50 percent of the spores germinated, and isolations made by planting 4-mm. disks cut from the speckled skin, and incubated at 40° to suppress bacterial growth, yielded 100 percent *Botrytis* cultures.

Infection occurred in the inoculated areas (fig. 5) in direct proportion to the period the pods were held at 32° F. (table 1).

FIGURE 5.—Infection caused by germinated *Botrytis* spores within the nonwounded, inoculated areas on bell peppers held at 32° F. for 16 days prior to inoculation and then at 55° for 10 days. (Cracks in the decayed skin developed during photographing.)

TABLE 1.—*Results from dusting* Botrytis *spores on nonwounded areas on bell peppers after various periods of holding at 32° F.*

Days at 32° F. prior to inoculation	Inoculated areas	Inoculated areas with active rots	Infections arrested	Inoculated areas	
				Dark speckled	Not changed
	Number	*Percent*	*Percent*	*Percent*	*Percent*
0	486	6	2	92	0
4	288	11	1	86	2
8	378	36	5	59	0
12	288	55	11	34	0
16	186	86	4	10	0
20	306	92	2	3	3

This was the most conclusive test that low, but nonfreezing, temperatures weaken pepper pods and provide the predisposing condition for gray mold rot.

U.S. GOVERNMENT PRINTING OFFICE: 1966 O—222-365